The B-9 extract separated on paper reacts with the reagent and gives a dark blue spot against a yellow background. In a very short time the background turns dark blue, and location of B-9 is difficult or impossible. This problem can be prevented by spraying immediately with 2 *N* ammonium acetate.

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[1] J. A. RIDDELL, H. A. HAGEMAN, C. M. J'ANTHONY AND W. L. HUBBARD, *Science*, 136 (1962) 391.
[2] L. P. BATJER, M. W. WILLIAMS AND G. C. MARTIN, *Proc. Wash. State Hort. Assoc.*, 59 (1963) 124.
[3] G. C. MARTIN, M. W. WILLIAMS AND L. P. BATJER, *Proc. Am. Soc. Hort. Sci.*, Rept. 84.
[4] J. A. ROMBERGER, *U.S. Dept. Agr., Forest Serv., Pacific Southwest Forest Range Expt. Sta.*, (1960).
[5] I. SMITH, *Chromatography*, Vol. 1, Interscience, New York, 1960.

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Countercurrent distribution of D-lyxose-1-^{14}C*

During the course of countercurrent distribution studies, we observed that D-lyxose-1-^{14}C migrated less rapidly than its unlabeled counterpart during countercurrent distribution. These data are similar to those previously reported[1] for D-arabinose-1-^{14}C and extend to these aldopentoses, during countercurrent distribution in cyclohexane–ethanol, PIEZ AND EAGLE'S[2] caution concerning the use of coincidence of radioactivity and an index of mass as the criterion for identity in studies of labeled amino acids. Implicit in such migration of solutes during countercurrent distribution as well as chromatography is the considerable error that can result in the selection of a single fraction rather than the peak for determinations of specific activity.

Materials

D-Lyxose-1-^{14}C and D-xylose-1-^{14}C with specific activities, respectively, of 0.18 and 0.21 mC per millimole were purchased from Calbiochem. The radiochemical purity of all compounds was found to be higher than 98 % when mass calculated from observed characteristic absorbance and absorbance index[3] was compared with mass computed from radioactivity and sample specific activity. *o*-Aminobiphenyl, purchased from Chemical Procurement Laboratories, College Point, New York, was purified by recrystallization.

Experimental

Countercurrent distribution. Twenty to 30 mg of a mixture of inert pentose and radioactive isomer with a final specific activity of 35–40 μC/mmole were dissolved in 100 ml of lower phase of the cyclohexane–ethanol system described in Fig. 1. The solution, after 24 h to permit anomeric equilibrium, was introduced into the first five tubes of the 100 tube countercurrent train. At the end of the indicated number of transfers, sampled tubes were dried in moving air at 22°. Dried samples were counted for

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radioactivity, then each residue was dissolved in 5 ml of water from which 1 ml was taken for the determination of the unlabeled sugar by measuring the absorbance at 370 mμ of the pentosylamine[3] formed in 30 min with *o*-aminobiphenyl.

As in the case of D-arabinose-1-^{14}C and L-arabinose-1-^{14}C, D-lyxose-1-^{14}C migrated less rapidly than unlabeled D-lyxose (Fig. 1) during countercurrent distribution for 600 transfers as well as for 950 transfers. The apparent distribution coefficient (0.11) at 950 transfers, calculated from the position of the tube having the maximum concentration of the pentose, was greater than the corresponding value (0.06) for 600 transfers but such would be expected since no compensation (by calculation or solvent replacement) was made for the gradually decreasing lower phase volumes after 600 transfers. There was no difference between the mobility of D-xylose-1-^{14}C and inert D-xylose in the cyclohexane–ethanol system (Fig. 2). No measurable radioactivity appeared in tubes 0 to 24 or between 60 and 100 when labelled xylose underwent the distribution shown. When the distribution was carried out through 400 transfers, no difference in mobility between the labeled and unlabeled pentose was observed.

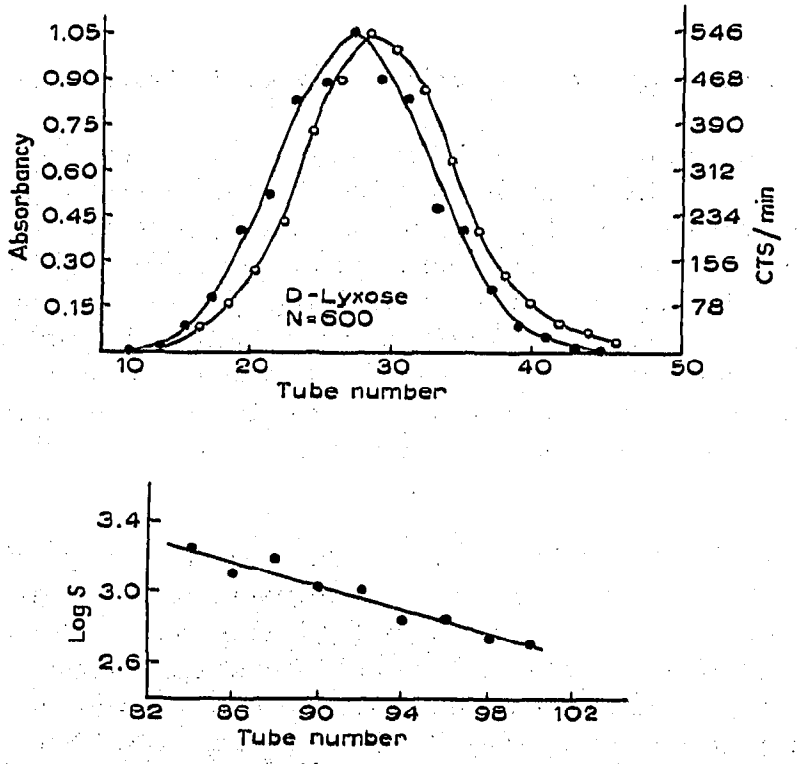

Fig. 1. (Upper) Resolution of D-lyxose-1-^{14}C and unlabeled D-lyxose in the all glass countercurrent apparatus of CRAIG AND POST with each phase volume 10 ml. The solvent system was an equilibrated mixture of 2 parts of cyclohexane with 1 part 95% ethanol at 22°. The upper phase composition, by volume in percent of water, ethanol and cyclohexane, was 0.8, 15.5 and 84.5 as determined by matching spectra of synthetic mixtures of the components using the Perkin-Elmer infrared spectrophotometer, Model 21. The corresponding composition of water, ethanol and cyclohexane in the lower phase was 2.5, 48 and 49: ● = radioactivity, measured with an end window Geiger counter; ○ = absorbance at 370 mμ of the arabinosylamine. Twenty mg of unlabeled D-lyxose mixed with 5 μC of labelled pentose constituted the sample for the 600 transfer distribution shown. (Lower) Plot of log specific activity, log S, against tube number, X, in accordance with $\ln S = [(M_1 - M_2)X/\sigma^2] + [(M_2^2 - M_1^2)/2\sigma^2]$ derived from the ratio of two curves (absorbance, ^{14}C-activity) assuming the normal distribution and that they have the same standard deviation σ, but M_1, the mean of the absorbance curve, differs from M_2, the mean of the ^{14}C activity curve; thus, the slope of the line is the index of resolution for this 950 transfer distribution.

Although the countercurrent distribution of xylose compares a 200 transfer (Fig. 2) extraction with a 600 transfer one (Fig. 1), the greater mobility of xylose (K = 0.26) compared to lyxose (K = 0.06) permits the comparison[4]. Indeed,

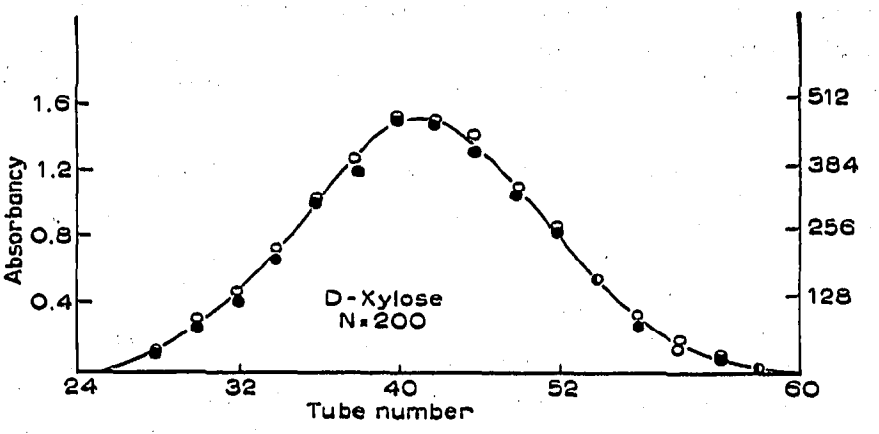

Fig. 2. Absorbancy at 370 millimicrons and radioactivity, a graphical representation of the coincidence of the mobility of D-xylose and D-xylose-1-^{14}C in a distribution similar to that described in Fig. 1 with the exception that the number of transfers was 200. ● = Radioactivity, measured with an end window Geiger counter. O = Absorbance, at 370 millimicrons of the xylosylamine.

if the distribution coefficients of D-xylose-1-^{14}C and inert D-xylose had the same ratio as the corresponding apparent distribution coefficients of D-lyxose-1-^{14}C and unlabeled D-lyxose (Fig. 1), separation of the peaks of the distribution patterns for radioactive and inert xylose would be greater at 200 transfers than that shown for the isotopic partners of D-lyxose (Fig. 1) for 600 transfers.

If our reasoning is correct, ^{14}C on carbon 1 alters the dipole moment of the radioactive lyxose and, thereby, the distribution coefficient of this aldopentose in the cyclohexane system. Possibly several forms (for example, the aldehyde and one or more ring forms) comprise the lyxose sample undergoing such extraction, but lyxose-1-^{14}C contributes to the less rapidly migrating components during countercurrent distribution. Since the isotope effect was not observed with similarly labeled radioactive xylose, it might be that the corresponding equilibria are too one-sided for the effect to be discernible in countercurrent distribution in cyclohexane–ethanol. In these studies, as in the previous one for arabinose, the slightly broader than theoretical[4] distribution for lyxose as well as the slight deviation from linearity of plots of specific activity *versus* fraction number (Fig. 1 — lower) are at least consistent with such polymorphism.

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[1] L. M. MARSHALL AND R. E. COOK, *J. Am. Chem. Soc.*, 84 (1962) 2647.
[2] K. A. PIEZ AND H. EAGLE, *Science*, 122 (1955) 969.
[3] R. E. TIMELL, C. P. J. GLAUDEMANS AND A. L. CURRIE, *Anal. Chem.*, 28 (1956) 1916.
[4] L. C. CRAIG AND D. CRAIG, in A. WEISSBERGER (Editor), *Technique of Organic Chemistry*, Interscience Publishers, Inc., New York, N.Y., Vol. III, Part. I, 1956, pp. 150–332.

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